Pr ject No.______
B k N .______

Clone The mutants in pTTQ19

Pag No.__

Plan: Delete 5'→3' exo activity of The Pol mutants (F→Y) and 3'-5'/F-Y

Digest pTTQ19 with Sph + SmaI. Clone The mutants at sphI and SmaI site. The digest with SphI / SmaI (HindIII) will put The pol in frame and thus, there was need for any other manipulation.

pTTQ19 : 5µl (0.5µg)
TE 20µl
React 4 3µl
SphI/Sma 1µl / 1µl
30' / 37°C. → freeze until other two are ready

pUC19FY : 60µl DNA
6µl React 2
2µl HindIII

pUC35FY (#2) : 60µl
6µl React 2
2µl HindIII

After 30' at 37°c add 5µl 1mM dNTP mix + 1µl (5U) Klenow — Incubate for 5' on ice. Add EDTA 2µl mM → EtOH ppt → see next page.

```
                -10                                      RBS       met asn ser arg gly ser val asp leu gln pro ser leu ala leu ala
AATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAAACAGCG ATG AAT TCC CGG GGA TCC GTC GAC CTG CAG CCA AGC TTG GCA CTG GCC   pTTQ8
                                                                       EcoRI ___ _BamHI  ___     PstI    HindIII
                                                                            SmaI          SalI

                -10                                      RBS       met ser leu ala ala gly arg arg ile pro gly asn ser leu ala
AATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAAACAGCG ATG AGC TTG GCT GCA GGT CGA CGG ATC CCC GGG AAT TCA CTG GCC       pTTQ9
                                                                              PstI  ___ _ BamHI ___ EcoRI
                                                                                  SalI      SmaI

                -10                                      RBS       met asn ser ser ser val pro gly asp pro leu glu ser thr cys arg his ala ser leu ala leu ala
AATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAAACAGCG ATG AAT TCG AGC TCG GTA CCC GGG GAT CCT CTA GAG TCG ACC TGC AGG CAT GCA AGC TTG GCA CTG GCC   pTTQ18 ✓
                                                                       EcoRI _ ___ __ KpnI___ ___ BamHI _ ___ __ SalI  _ ___ __ SphI
                                                                              SstI      SmaI          XbaI          PstI        HindIII

                -10                                      RBS       met ser leu his ala cys arg ser thr leu glu asp pro arg val pro ser ser asn ser leu ala
AATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAAACAGCG ATG AGC TTG CAT GCC TGC AGG TCG ACT CTA GAG GAT CCC CGG GTA CCG AGC TCG AAT TCA CTG GCC   ← pTTQ19
                                                                              SphI _ ___ __ SalI _ ___ __ BamHI___ ___ KpnI _ ___ __ EcoRI
                                                                                   PstI             XbaI       SmaI           SstI

                -10                                      RBS       met asn leu ile thr asn ser ser ser val pro gly asp pro leu glu ser thr cys arg his ala ser leu
AATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAAACAGCG ATG AAT TTG ATT ACG AAT TCG AGC TCG GTA CCC GGG GAT CCT CTA GAG TCG ACC TGC AGG CAT GCA AGC TTG   pTTQ181
                                                                                   Eco RI _ ___ __ KpnI___ ___ BamHI _ ___ SalI _ ___ __ SphI _ ___ __
                                                                                          SstI       SmaI         XbaI         PstI          HindIII

                -10                                      RBS       met thr met ile thr asn ser ser ser val pro gly asp pro leu glu ser thr cys arg his ala ser leu
TATGCTTCCGGCTCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAAACAGCT ATG ACC ATG ATT ACG AAT TCG AGC TCG GTA CCC GGG GAT CCT CTA GAG TCG ACC TGC AGG CAT GCA AGC TTG   pUC18
                                                                                   EcoRI _ ___ __ KpnI___ ___ BamHI _ _ __ __SalI/AccI ___ __ SphI _ ___ __
                                                                                         SstI       SmaI          XbaI            PstI           HindIII
```

..otide sequences of the promoter and polylinker regions of the pTTQ vectors and pUC18. Sequence extending from the −35 region of the *lac* or *tac* promoter to the dist.. polylinker is given for pTTQ8, 9, 18, 19 and 181. The comparable region of pUC18 is also shown. Unique cloning sites in the polylinker, the −35 and −10 regions of th.. d the RBS are shown.

[signature] | 8/1/95 | Recorded by [signature] | 8/9/95 |

[redacted]

Project No.
40 Book No. TITLE The clones in pTTQ19 (4'-5'-exo)

From Page No.

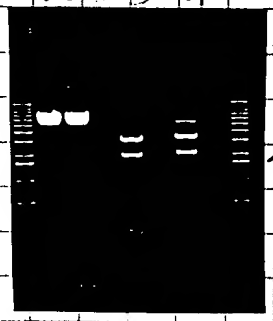
pUC Tne FY HindIII → blunt ended fragment → dissolve in 17µl T

pUC Tne 35FY HindIII → blunt ended fragment → dissolve in 17µl T

Sph I digestion

	FY	35 FY
DNA	17µl	17µl
React 6	2µl	2µl
Sph I	1µl	1µl

30 min / 37°c → Run gel.



#1 & #2 → pTTQ19 sph / sma

#3 → pUC Tne FY

#4 → pUC Tne 35 FY

purify Vector & insert as a mixture by gene clean in H_2O.

ⓐ pTTQ19 + 2.0 Kb (Tne FY)

ⓑ pTTQ19 + 2.0 Kb (Tne 35FY)

ligation

15µl DNA mix
4µl 5 x buffer
1µl SU ligase

Ligate for 15 min at room temp.

Transform DH10B. Plate 10% & 90% culture – 30°C /ON.

To Pag No

Witnessed & Understood by me,	Date	Invented by	Date
	8/7/95	Rcorded by	7/20/95

Tne clones in pTTQ19. (Δ 5'-exo)

Proj ct No.

B ok N .

Pag No.

Result of transformation:

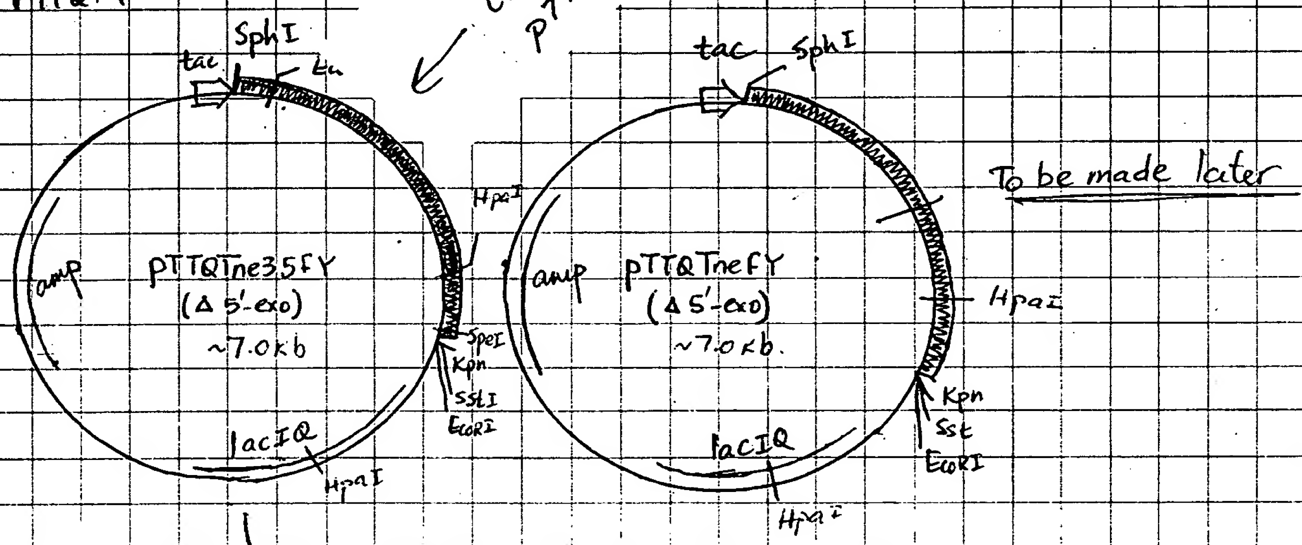
pTTQ Tne 35/FY :	10%	105
	90%	TMTC
pTTQ Tne FY	10%	115
	90%	TMTC.

Left the plates in the bench over the week end.

Inoculate 6 clones from for mini prep (2 mL EG/Amp).
Overnight growth at 30°C.

Standard mini prep! was dissolve in 150μl TE.
Digest clones with SstI. The SstI will be coming from pTTQ19.

Listed as "pTTQ Tne 535FY in App."



Please see Mary Longo's note book #57 (LTI Book #3959, p183).

To Page No.

ssed & Und rstood by m ,	Dat	Invent d by	Date
[signature]	8/7/95	Recorded by Deb [signature]	7/21/95